LIGAND-ACTIVATABLE FLUORESCENT PROTEIN FOR SINGLE MOLECULE LOCALIZATION MICROSCOPY

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Most fluorescent proteins have covalently linked chromophore that can not be replaced after damaged by photobleaching. In contrast, UnaG, a ligand-activatable fluorescent protein, encapsulates the fluorogenic ligand, bilirubin, via interaction network composed many hydrogen bondings between the chromophore and the protein. We found that the noncovalent linkage allow for detachment of the photobleached ligand that empties the binding cavity for rebinding to a new undamaged ligand in solution. The reaction cycle of photobleaching via photooxidation, unbinding and rebinding can be repeated in micromolar concentration of the ligands in solution. We used the reversible dark-to-green photoswitching for photobleaching-resistant single-molecule localization microscopy (SMLM). Since the nonfluorescent ligands in solution that do not add in fluorescent background, we could achieve photobleaching-resistant SMLM in standard Epi illumination.